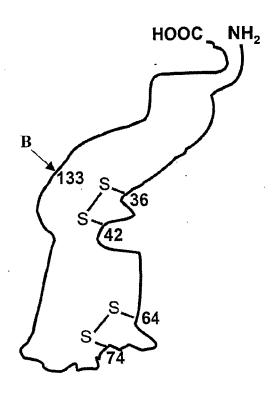
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$$\mathbf{B} \leftarrow \begin{pmatrix} (\operatorname{Sia})_{b} \\ -\operatorname{GalNAc-(Gal)}_{a} - (\operatorname{Sia})_{c} - (R)_{d} \end{pmatrix}_{e}$$

a-c, e (independently selected) = 0 or 1;
d = 0;
R = modifying group, sialyl or oligosialyl

FIG. 29A

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CHO, BHK, 293 cells, Vero expressed G-CSF a-c, e (independently selected) = 0 or 1; d = 0

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1; R = PEG.

FIG. 29B

Insect cell expressed G-CSF a, e (independently selected) = 0 or 1; b, c, d = 0.

- ↓ 1. Galactosyltransferase, UDP-Gal2. CMP-SA-PEG, ST3Gal1
- a, c, d, e (independently selected) = 0 or 1; R = PEG.

FIG. 29C

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E. coli expressed G-CSF a-e=0.

- 1. GalNAc Transferase, UDP-GalNAc▼ 2. CMP-SA-PEG, sialyltransferase
- c, d, e (independently selected) = 0 or 1; a, b = 0; R = PEG.

FIG. 29D

NSO expressed G-CSF a, e (independently selected) = 0 or 1; b, c, d = 0

1. CMP-SA-levulinate, ST3Gal1
 2. H₄N₂-PEG

a, c, d, e (independently selected) = 0 or 1; b = 0; R = PEG.

FIG. 29E

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E. coli expressed G-CSF a-e = 0.

1. Endo-GalNAc-osaminidase (synthetic enzyme), PEG-Gal-GalNAc-F

a, d, e = 1; b, c = 0; R = PEG.

FIG. 29F

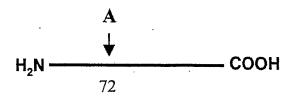
E. coli expressed G-CSF a-e = 0.

1. GalNAc Transferase, UDP-GalNAc-PEG

d, e = 0 or 1; a,-c = 0; R = PEG.

FIG. 29G

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$$\mathbf{A} \leftarrow \begin{bmatrix} \left[\operatorname{GlcNAc-(Gal)}_{a} \right]_{e} - \left(\operatorname{Sia} \right)_{j} - \left(\operatorname{R} \right)_{v} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{b} \right]_{f} - \left(\operatorname{Sia} \right)_{k} - \left(\operatorname{R} \right)_{w} \right]_{s} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{b} \right]_{g} - \left(\operatorname{Sia} \right)_{l} - \left(\operatorname{R} \right)_{x} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} \right]_{u} = aa} \end{bmatrix}_{bb}$$

a-d, i, n-u (independently selected) = 0 or 1.

aa, bb, cc, dd, ee (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 20.

v-z = 0; R = modifying group, mannose, oligo-mannose.

R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 30A

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```
CHO, BHK, 293 cells, Vero expressed interferon alpha 14C.

a-d, aa, bb = 1; e-h = 1 to 4; cc, j-m, i, r-u (independently selected) = 0 or 1; q, n-p, v-z, cc, dd, ee = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal3

```
a-d, aa, bb = 1; e-h = 1 to 4;
bb, cc, i, r-u (independently selected) = 0 or 1;
q, n-p, v-z, cc, dd, ee = 0;
v-y (independently selected) = 1,
when j-m (independently selected) = 1;
R = PEG.
```

FIG. 30B

```
Insect cell or fungi expressed interferon alpha-14C. a-d, f, h, j-q, s, u, v-z, cc, dd, ee = 0; e, g, i, r, t (independently selected) = 0 or 1; aa, bb = 1.
```

- 1. GNT's 1&2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
b, d, f, h, j-q, s, u, w, y, z, cc, dd, ee = 0;
a, c, e, g, i, r, t, v, x (independently selected) = 0 or 1;
v, x (independently selected) = 1,
when a, c, (independently selected) = 1;
aa, bb = 1; R = PEG.
```

FIG. 30C

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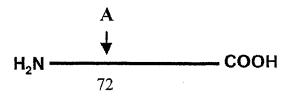
```
Yeast expressed interferon alpha-14C.
a-q, cc, dd, ee, v-z = 0;
r-y (independently selected) = 0 to 1;
aa, bb = 1;
R (branched or linear) = Man, oligomannose or polysaccharide.
```

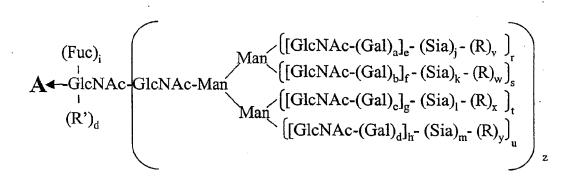
- 1. Endo-H
- 2. Galactosyltransferase, UDP-Gal
- 3.. CMP-SA-PEG, ST3Gal3

a-z, bb = 0; aa = 1; R' = -Gal-Sia-PEG.

FIG. 30D

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a-d, i, r-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 or 1. n, v-y = 0; z = 0 or 1. R = polymer; R' = sugar, glycoconjugate.

FIG. 30E

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```
CHO, BHK, 293 cells, Vero expressed interferon alpha-14C.

h = 1 to 3;

a-g, j-m, i (independently selected) = 0 or 1;

r-u (independently selected) = 0 or 1;

n, v-y = 0; z = 1.
```

1. CMP-SA-PEG, ST3Gal3

```
h = 1 to 3;
a-g, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.
```

FIG. 30F

```
Insect cell or fungi expressed
interferon alpha-14C.
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, r, t (independently selected) = 0 or 1;
z = 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1; z = 1; n = 0; R = PEG.

FIG. 30G

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Yeast expressed interferon alpha-14C. a-n = 0; r-y (independently selected) = 0 to 1; z = 1; R (branched or linear) = Man, oligomannose.

- 1. mannosidases
- 2. GNT's 1,2,4,5, UDP-GlcNAc
- 3. Galactosyltransferase, UDP-Gal
- 4.. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1; z = 1; n = 0; R = PEG.

FIG. 30H

NSO expressed interferon alpha 14C. a-i, r-u (independently selected) = 0 or 1; j-m, n, v-y = 0; z = 1.

- 1. CMP-SA-levulinate, ST3Gal3, buffer, salt
- 2. H₄N₂-PEG

a-i, j-m, r-y (independently selected) = 0 or 1; n = 0; z = 1; R = PEG.

FIG. 301

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CHO, BHK, 293 cells, Vero expressed interferon alpha-14C.

h = 1 to 3;

a-g, j-m, i (independently selected) = 0 or 1;

r-u (independently selected) = 0 or 1;

n, v-y = 0; z = 1.

1. CMP-SA-PEG, α 2,8-ST

h = 1 to 3; a-g, i, r-u (independently selected) = 0 or 1; j-m (independently selected) = 0 to 2; v-y (independently selected) = 1, when j-m (independently selected) is 2; z = 1; n = 0; R = PEG.

FIG. 30J

CHO, BHK, 293 cells, Vero expressed Interferon alpha-14C.
a-g, j-m, r-u (independently selected) = 0 or 1; h = 1 to 3; n, v-y = 0; z = 1.

- 1. Sialidase
- 2. Trans-sialidase, PEG-Sia-lactose

a-g, j-m, r-y (independently selected) = 0 or 1; h = 1 to 3; n = 0; z = 1; R = PEG.

FIG. 30K

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```
CHO, BHK, 293 cells, Vero expressed interferon alpha-14C.

h = 1 to 3;

a-g, j-m, i (independently selected) = 0 or 1;

r-u (independently selected) = 0 or 1;

n, v-y = 0; z = 1.
```

1. CMP-SA, α 2,8-ST

```
h = 1 to 3;
a-g, i, r-u (independently selected) = 0 or 1;
j-m (independently selected) = 0 to 40;
z = 1; v-y, n = 0.
```

FIG. 30L

Insect cell or fungi expressed interferon alpha-14C. a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t (independently selected) = 0 or 1; z = 1.

- 1. GNT's 1 & 2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-linker-SA-CMP
- 3. ST3Gal3, transferrin

a, c, e, g, i, r, t, v, x (independently selected) = 0 or 1; z = 1; b, d, f, h, j-n, s, u, w, y = 0; R = transferrin.

FIG. 30M

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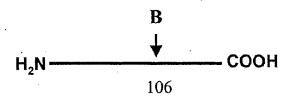
```
Insect cell or fungi expressed interferon alpha-14C.
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, r, t (independently selected) = 0 or 1; z = 1.
```

- 1. endoglycanase
- 2. Galactosyltransferase, UDP-Gal-linker-SA-CMP
- 3. ST3Gal3, transferrin

```
i (independently selected) = 0 or 1;
a-h, j-m, r-z = 0;
n = 1; R' = -Gal-linker-transferrin.
```

FIG. 30N

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$$\mathbf{B} \leftarrow \begin{bmatrix} (\operatorname{GlcNAc-Gal})_{f_{\underline{-}}}(\operatorname{Sia})_{b} - (R)_{g} \\ -\operatorname{GalNAc-(Gal})_{a} - (\operatorname{Sia})_{c} - (R)_{d} \end{bmatrix}_{e}$$

a-c, e, f (independently selected) = 0 or 1; d, g = 0; R = polymer, glycoconjugate.

FIG. 300

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CHO, BHK, 293 cells, Vero expressed IF-alpha (2a or 2b). a-c (independently selected) = 0 or 1; e = 1; d, f, g = 0

1. Sia

1. Sialidase

2. CMP-SA-PEG, ST3Gal1

a-d (independently selected) = 0 or 1; e = 1; b, f, g = 0; R = PEG.

FIG. 30P

Insect cell expressed interferon alpha (2a or 2b). a, e (independently selected) = 0 or 1; b, c, d, f, g = 0.

- 1. Galactosyltransferase, UDP-Gal
- 2. CMP-SA-PEG, ST3Gal1

a, c, d, e (independently selected) = 0 or 1; b, f, g = 0; R = PEG.

FIG. 30Q

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E. coli expressed IF-alpha (2a or 2b). a-g=0.

 GalNAc Transferase, UDP-GalNAc-PEG

a-c, f, g = 0; d, e = 1; R = PEG.

FIG. 30R

NSO expressed IF-alpha (2a or 2b). a (independently selected) = 0 or 1; e = 1; b, c, d, f, g = 0

- 1. CMP-SA-levulinate, ST3Gal1
- $2. H_4N_2$ -PEG

a, c, d (independently selected) = 0 or 1; e = 1; b, f, g = 0; R = PEG.

FIG. 30S

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E. coli expressed IF-alpha (2a or 2b). a-g=0.

 Endo-N-acetylgalatosamidase (synthetic enzyme), PEG-Gal-GalNAc-F

a, d, e = 1; b, c, f, g = 0; R = PEG.

FIG. 30T

E. coli expressed IF-alpha (2a or 2b). a-g=0.

- 1. GalNAc Transferase, UDP-GalNAc
- 2. sialyltransferase, CMP-SA-PEG

b, d = 0 or 1; e = 1; a, c, f, g = 0; R = PEG.

FIG. 30U

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CHO, BHK, 293 cells, Vero expressed IF-alpha (2a or 2b).

a-c, f (independently selected) = 0 or 1;

e = 1; d, g = 0

Sialidase

2. CMP-SA-PEG, ST3Gal1 and ST3Gal3

a-d, f, g (independently selected) = 0 or 1; e = 1; R = PEG.

FIG. 30V

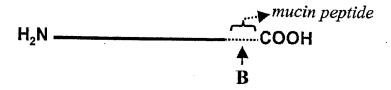
CHO, BHK, 293 cells, Vero expressed IF-alpha (2a or 2b). a-c, f (independently selected) = 0 or 1; e = 1; d, g = 0

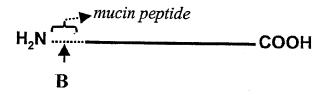
- 1. Sialidase
- 2. CMP-SA-linker-SA-CMP, ,ST3Gal1
- 3. ST3Gal3, transferrin

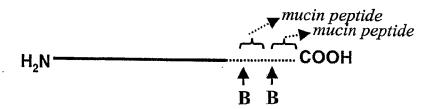
a-d, f (independently selected) = 0 or 1; e = 1; R = transferrin; g = 0.

FIG. 30W

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$$\mathbf{B} \leftarrow \begin{pmatrix} (\operatorname{Sia})_{b} \\ -\operatorname{GalNAc-(Gal)}_{a} - (\operatorname{Sia})_{c} - (\operatorname{R})_{d} \end{pmatrix}_{e}$$

a-c, e (independently selected) = 0 or 1; d = 0; R = polymer, glycoconjugate.

FIG. 30X

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CHO, BHK, 293 cells, Vero expressed interferon alpha-mucin fusion protein. a-c, e (independently selected) = 0 or 1; d = 0

- 1. Sialidase
 - 2. CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1; R = PEG.

FIG. 30Y

Insect cell expressed interferon alpha-mucin fusion protein.

a, e (independently selected) = 0 or 1; b, c, d = 0.

1. Galactosyltransferase, UDP-Gal-PEG

a, d, e (independently selected) = 0 or 1; b, c = 0; R = PEG.

FIG. 30Z

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E. coli expressed interferon alpha-mucin fusion protein.

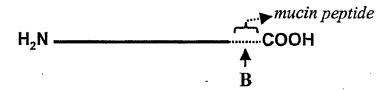
a-e = 0.

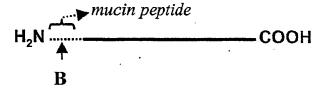
- 1. GalNAc Transferase, UDP-GalNAc
- 2. CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1; a, b = 0; R = PEG.

FIG. 30AA

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$$\mathbf{B} \leftarrow \begin{pmatrix} (\operatorname{Sia})_{b} \\ -\operatorname{GalNAc-(Gal)}_{a} - (\operatorname{Sia})_{c} - (R)_{d} \end{pmatrix}_{e}$$

$$\mathbf{C} \leftarrow (\mathbf{R'})_{\mathbf{n}}$$

a-c, e (independently selected) = 0 or 1; d = 0; R = polymer, linker.

FIG. 30BB

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E. coli expressed interferon alpha-mucin fusion protein.

a-e, n = 0.

1. GalNAc Transferase, UDP-GalNAc-PEG

d, e (independently selected) = 0 or 1; a-c, n = 0; R = PEG.

FIG. 30CC

E. coli expressed interferon alpha-mucin fusion protein.

a-e, n = 0.

- GalNAc Transferase, UDP-GalNAc-linker-SA-CMP
- 2. ST3Gal3, asialo-transferrin
- 3. CMP-SA, ST3Gal3

d, e (independently selected) = 0 or 1; a-c, n = 0; R = linker-transferrin.

FIG. 30DD

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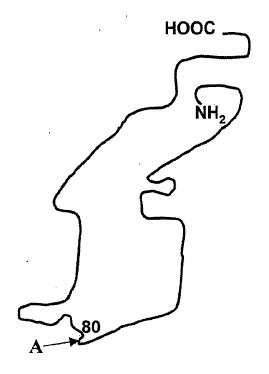
E. coli expressed Interferon alpha (no fusion). a-e, n = 0.

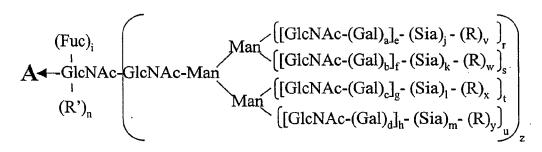
- 1. NHS-CO-linker-SA-CMP
- 2. ST3Gal3, transferrin

a-e=0; n=1; R' = linker-transferrin.

FIG. 30EE

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a-d, i, r-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 or 1. n, v-y = 0; z = 0 or 1; R = polymer

FIG. 31A

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CHO, BHK, 293 cells, Vero expressed IF-beta h = 1 to 3: a-g, j-m, i (independently selected) = 0 or 1: r-u (independently selected) = 0 or 1; n, v-y = 0; z = 1.

1. Sialidase

2. CMP-SA-PEG, ST3Gal3

```
h = 1 \text{ to } 3;
a-g, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.
```

FIG. 31B

```
Insect cell expressed IF-beta
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, r, t (independently selected) = 0 or 1;
z = 1.
```

- 1. GNT's 1&2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- 2. CMP-SA-PEG, ST3Gal3, buffer, salt

b, d, f, h, k, m, n, s, u, w, y = 0; a, c, e, g, i, r, t (independently selected) = 0 or 1; j, l, v, x (independently selected) = 0 or 1: z = 1; R = PEG.

FIG. 31C

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Yeast expressed IF-beta a-n = 0; z = 1; r-y (independently selected) = 0 to 1; R (branched or linear) = Man, oligomannose or polysaccharide.

- 1. Endo-H
- 2. Galactosyltransferase, UDP-Gal
- 3.. CMP-SA-PEG, ST3Gal3

```
a-m, r-z=0; n=1; R'=-Gal-Sia-PEG.
```

FIG. 31D

```
CHO, BHK, 293 cells, Vero expressed IF-beta h = 1 to 3; a-g, j-m, i (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; n, v-y = 0; z = 1.
```

1. CMP-SA-PEG, ST3Gal3

```
h = 1 to 3;
a-g, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.
```

FIG. 31E

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Insect cell expressed IF-beta a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t (independently selected) = 0 or 1; z = 1.

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1; z = 1; n = 0; R = PEG.

FIG. 31F

Yeast expressed IF-beta a-n = 0; z = 1; r-y (independently selected) = 0 to 1; R (branched or linear) = Man, oligomannose.

- 1. mannosidases
- 2. GNT's 1,2,4,5, UDP-GlcNAc
- 3. Galactosyltransferase, UDP-Gal
- 4.. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1; z = 1; n = 0; R = PEG.

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NSO expressed IF-beta a-i, r-u (independently selected) = 0 or 1; j-m, n, v-y = 0; z = 1.

> CMP-SA-levulinate, ST3Gal3, buffer, salt
> H₄N₂-PEG

a-i, j-m, r-y (independently selected) = 0 or 1; n = 0; z = 1; R = PEG.

FIG. 31H

CHO, BHK, 293 cells, Vero expressed IF-beta h = 1 to 3; a-g, j-m, i (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; n, v-y = 0; z = 1.

1. CMP-SA-PEG, α 2,8-ST

h = 1 to 3;
a-g, i, r-u (independently selected) = 0 or 1;
j-m (independently selected) = 0 to 2;
v-y (independently selected) = 1,
when j-m (independently selected) is 2;
z = 1; n = 0; R = PEG.

FIG. 311

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CHO, BHK, 293 cells, Vero expressed IF-beta a-g, j-m, r-u (independently selected) = 0 or 1; h = 1 to 3; n, v-y = 0; z = 1.

- 1. Sialidase
- 2. Trans-sialidase, PEG-Sia-lactose

a-g, j-m, r-y (independently selected) = 0 or 1; h = 1 to 3; n = 0; z = 1; R = PEG.

FIG. 31J

CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n, v-y = 0.

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n=0; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 31K

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```
NSO expressed Ifn-beta.

a-d, i-m, r-u, z (independently selected) = 0 or 1;

e-h = 1; n, v-y = 0;

Sia (independently selected) = Sia or Gal.

1. Sialidase and α-galactosidase
2. α-Galactosyltransferase, UDP-Gal

3. CMP-SA-PEG, ST3Gal3

a-d, i-m, r-u, z (independently selected) = 0 or 1;

e-h = 1; R = PEG

n = 0; v-y (independently selected) = 1,

when j-m (independently selected) is 1;
```

FIG. 31L

```
CHO, BHK, 293 cells, Vero expressed Ifn-beta.
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; n, v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

```
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; n = 0;
v-y (independently selected) = 0 or 1; R = PEG.
```

FIG. 31M

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CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h=1; n, v-y=0.

- 1. CMP-SA-levulinate, ST3Gal3, buffer, salt
- 2. H_4N_2 -PEG

```
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; n = 0;
v-y (independently selected) = 0 or 1; R = PEG.
```

FIG. 31N

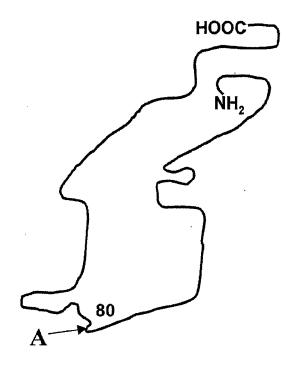
```
CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n, v-y = 0.
```

1. CMP-SA, α2,8-ST

a-d, i, r-u, z (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; n, v-y (independently selected) = 0.

FIG. 310

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$$\mathbf{A} \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} & & & & \\ (\operatorname{GlcNAc-(Gal)}_{a}]_{e} - (\operatorname{Sia})_{j} - (\operatorname{R})_{v} \\ (\operatorname{GlcNAc-Man} & & & & \\ (\operatorname{GlcNAc-(Gal)}_{b}]_{f} - (\operatorname{Sia})_{k} - (\operatorname{R})_{w} \end{bmatrix}_{s} \\ \operatorname{Man} = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)}_{b}]_{g} - (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ (\operatorname{R'})_{n} & & & & \\ (\operatorname{GlcNAc-(Gal)}_{d}]_{h} - (\operatorname{Sia})_{m} - (\operatorname{R})_{y} \end{bmatrix}_{u} \\ \operatorname{GlcNAc-(Gal)}_{d} = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)}_{b})_{l} - (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ (\operatorname{GlcNAc-(Gal)}_{d})_{l} - (\operatorname{Sia})_{m} - (\operatorname{R})_{y} \end{bmatrix}_{u} \\ \operatorname{GlcNAc-(Gal)}_{d} = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)}_{b})_{l} - (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ (\operatorname{GlcNAc-(Gal)}_{d})_{l} - (\operatorname{Sia})_{m} - (\operatorname{R})_{v} \end{bmatrix}_{u} \\ \operatorname{GlcNAc-(Gal)}_{d} = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)}_{b})_{l} - (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ (\operatorname{GlcNAc-(Gal)}_{d})_{l} - (\operatorname{Sia})_{m} - (\operatorname{R})_{v} \end{bmatrix}_{u}$$

a-d, i, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group; R' = H, glycosyl group, modifying group, glycoconjugate.

FIG. 31P

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```
Insect cell expressed Ifn-beta.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 31Q

```
Yeast expressed Ifn-beta.

a-m = 0; q-y (independently selected) = 0 to 1;

p = 1;

R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- ▼ 3. CMP-SA-PEG, ST3Gal3

```
a-m, p-y = 0;
n (independently selected) = 0 or 1;
R' = -Gal-Sia-PEG.
```

FIG. 31R

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CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- CMP-SA-linker-SA-CMP, ST3Gal3
 ST3Gal3, desialylated transferrin.
- 3. CMP-SA, ST3Gal3

```
a-m, q-u (independently selected) = 0 or 1;

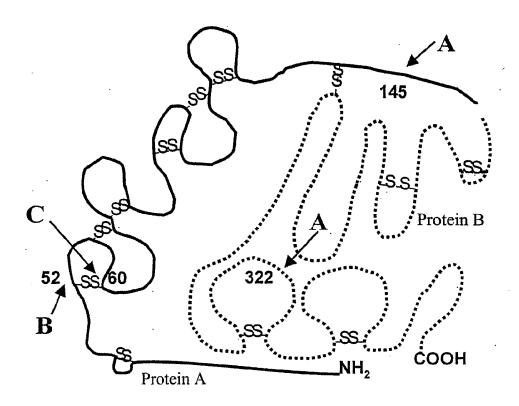
p = 1; n = 0;

v-y (independently selected) = 0 or 1;

R = linker-transferrin.
```

FIG. 31S

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$$\mathbf{A} \leftarrow \begin{bmatrix} \left[\operatorname{GlcNAc-(Gal)}_{a} \right]_{e} - \left(\operatorname{Sia} \right)_{j} - \left(\operatorname{R} \right)_{v} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{b} \right]_{f} - \left(\operatorname{Sia} \right)_{k} - \left(\operatorname{R} \right)_{w} \right]_{s} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{c} \right]_{g} - \left(\operatorname{Sia} \right)_{l} - \left(\operatorname{R} \right)_{x} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{q} \\$$

$$\mathbf{B} \blacktriangleleft \left(\mathsf{Glc}\text{-}(\mathsf{Xyl})_{\mathsf{n}} \right)_{\mathsf{o}}$$

a-d, i, q-u (independently selected) = 0 or 1. o, p (independently selected) = 0 or 1. e-h, n (independently selected) = 0 to 6. j-m (independently selected) = 0 to 20. v-y = 0; R = modifying group, mannose, oligo-

mannose, Sia-Lewis X, Sia-Lewis A..

FIG. 32A

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BHK expressed Factor VII or VIIa a-d, e, i, g, q, j, l, o, p (independently selected) = 0 or 1; r, t = 1; f, h, k, m, s, u, v-y = 0; n = 0-4.

- 1. Sialidase
 - 2. CMP-SA-PEG (16 mole eq), ST3Gal3

```
a-d, e, g, i, q, j, l, o, p (independently selected) = 0 or 1;

r, t = 1; f, h, k, m, s, u, w, y = 0; n = 0-4;

v, x, (independently selected) = 1,

when j, l (respectively, independently selected) is 1;

R = PEG.
```

FIG. 32B

CHO, BHK, 293 cells, Vero expressed Factor VII or VIIa a-d, e, i, g, q, j, l, o, p (independently selected) = 0 or 1; r, t = 1; f, h, k, m, s, u, v-y=0; n=0-4.

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mole eq), ST3Gal3
- 3. CMP-SA (8 mol eq), ST3Gal3

```
a-d, e, g, i, q, j, l, o, p (independently selected) = 0 or 1;
r, t = 1; f, h, k, m, s, u, w, y = 0; n = 0-4;
v or x, (independently selected) = 1,
when j or l, (respectively, independently selected) is 1;
R = PEG.
```

FIG. 32C

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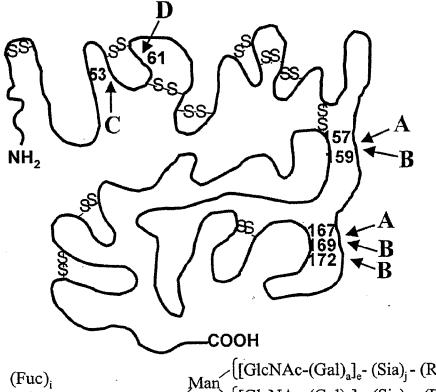
```
NSO expressed Factor VII or VIIa
a--u (independently selected) = 0 or 1;
v-y = 0; n = 0-4;
Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α -galactosidase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

```
a-m, o-u (independently selected) = 0 or 1;
n = 0-4; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
Sia = Sia; R = PEG.
```

FIG. 32D

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$$(Fuc)_{i} \qquad \qquad ([GlcNAc-(Gal)_{a}]_{e}-(Sia)_{j}-(R)_{v})_{r} \\ ([GlcNAc-(Gal)_{b}]_{f}-(Sia)_{k}-(R)_{w})_{s} \\ ([GlcNAc-(Gal)_{c}]_{g}-(Sia)_{l}-(R)_{x})_{t} \\ ([GlcNAc-(Gal)_{d}]_{h}-(Sia)_{m}-(R)_{y})_{u} \\ (GlcNAc-(Gal)_{d}]_{h}-(Sia)_{m}-(R)_{y})_{u} \\ (GlcNAc-(Gal)_{d}]_{h}-(Sia)_{m}-(R)_{y} \\ (GlcNAc-(Gal)_{d})_{u} \\ (GlcNAc-(G$$

$$\mathbf{B} \leftarrow \begin{bmatrix} (\operatorname{Sia})_{o} \\ -\operatorname{GalNAc-(Gal)}_{n} - (\operatorname{Sia})_{p} - (\operatorname{R})_{z} \end{bmatrix}_{q} \qquad \mathbf{C} \leftarrow \begin{bmatrix} -\operatorname{Glc-(Xyl)}_{aa} \end{bmatrix}_{bb}$$

$$\mathbf{D} \leftarrow \text{-Fuc}\left\{-(\text{GlcNAc})_{\text{cc}} - (\text{Gal})_{\text{dd}} - (\text{Sia})_{\text{ee}}\right\}_{\text{ff}} - (R)_{\text{gg}}$$

a-d, i, n-u (independently selected) = 0 or 1.
bb, cc, dd, ee, ff, gg (independently selected) = 0 or 1.
e-h, aa (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 20.
v-z = 0; R = modifying group, mannose, oligo-mannose.

FIG. 33A

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```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal3

```
a-d, q = 1; e-h = 1 to 4;

aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)

= 0 or 1;

o, p, z = 0;

j-m, ee, v-y, gg (independently selected) = 0 or 1;

R = PEG.
```

FIG. 33B

```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, n, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal3
- 3. ST3Gal1, CMP-SA

```
a-d, n, p, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, r-u (independently selected) = 0 or 1;
j-m, ee, v-y, gg (independently selected) = 0 or 1;
o, z = 0; R = PEG.
```

FIG. 33C

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CHO, BHK, 293 cells, Vero expressed Factor IX a-d, n, q, bb, cc, dd, ff = 1; e-h, aa = 1 to 4; ee, j-m, i, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.

- 1. sialidase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA, ST3Gal3
- 4. CMP-SA-PEG, ST3Gal1

```
a-d, n, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, r-u (independently selected) = 0 or 1; R = PEG;
o, v-y, gg = 0;
j-m, p, ee (independently selected) = 0 or 1, but when p = 1, z = 1.
```

FIG. 33D

```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.
```

CMP-SA-PEG, ST3Gal3

```
a-d, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)
= 0 or 1; R = PEG;
o, p, z = 0; j-m, ee, v-y, gg (independently selected) = 0 or 1.
```

FIG. 33E

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```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.
```

1. CMP-SA-levulinate, ST3Gal3, buffer, salt

2. H₄N₂-PEG

```
a-d, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)
= 0 or 1;
o, p, z = 0; R = PEG;
j-m, ee, v-y, gg (independently selected) = 0 or 1.
```

FIG. 33F

```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, n, q, bb, cc, dd, ff = 1; e-h, aa = 1 to 4; ee, j-m, i, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.
```

1. CMP-SA-PEG, α 2,8-ST

```
a-d, q = 1; e-h = 1 to 4;

aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)

= 0 or 1;

o, p, z = 0; R= PEG;

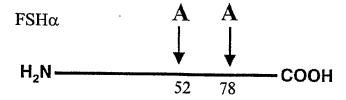
j-m, ee (independently selected) = 0 to 2;

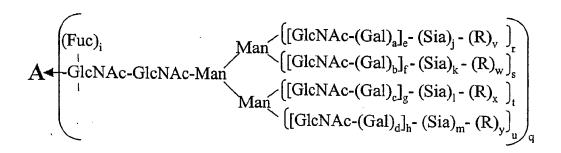
v-y, gg (independently selected) = 1, when j-m

(independently selected) is 2;
```

FIG. 33G

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a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose.

FIG. 34A

103/498

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.

FIG. 34B

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 34C

104/498

NSO expressed FSH.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0;

Sia (independently selected) = Sia or Gal.

- 1. Sialidase and α-galactosidase
- 2. Galactosyltransferase, UDP-Gal
- **★** 3. CMP-SA-PEG, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.

FIG. 34D

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 34E

105/498

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. CMP-SA-levulinate, ST3Gal3, buffer, salt
 - 2. H₄N₂-PEG

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 34F

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 34G

106/498

```
Insect cell expressed FSH.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 34H

```
Yeast expressed FSH.

a-m=0; q-y (independently selected) = 0 to 1;

p=1;

R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- ▼ 3. CMP-SA-PEG, ST3Gal3

```
a-m, p-y = 0;
n (independently selected) = 0 or 1;
R' = -Gal-Sia-PEG.
```

FIG. 341

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CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. CMP-SA-linker-SA-CMP, ST3Gal3
- 2. ST3Gal1, desialylated chorionic gonadrophin (CG) produced in CHO.
- 3. CMP-SA, ST3Gal3, ST3Gal1

```
a-m, q-u (independently selected) = 0 or 1;

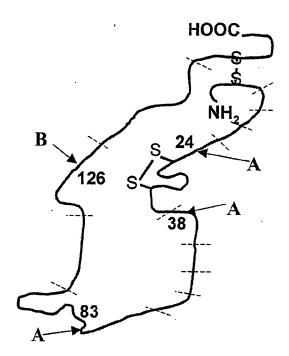
p = 1; n = 0;

v-y (independently selected) = 0 or 1;

R = linker-CG.
```

FIG. 34J

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(Fuc)_i

A -GlcNAc-GlcNAc-Man

$$\begin{bmatrix}
[GlcNAc-(Gal)_a]_e - (Sia)_j - (R)_v \\
[GlcNAc-(Gal)_b]_f - (Sia)_k - (R)_w \\
Man
\begin{bmatrix}
[GlcNAc-(Gal)_b]_f - (Sia)_k - (R)_w \\
Man
\end{bmatrix}_t \\
[[GlcNAc-(Gal)_c]_g - (Sia)_l - (R)_x \\
[[GlcNAc-(Gal)_d]_h - (Sia)_m - (R)_y \\
u
\end{bmatrix}_u$$

B -GalNAc-(Gal)_n-(Sia)_p- (R)_z
_q

a-d, i, n-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 to 20.

v-z = 0;

R = polymer.

FIG. 35A

109/498

```
CHO, BHK, 293 cells, Vero expressed EPO a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 to1; v-z = 0
```

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal3

```
a-g, n, q = 1; h = 1 to 3;
i, o, p (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
R = PEG; z = 0.
```

FIG. 35B

```
Insect cell expressed EPO
a-d, f, h, j-q, s, u, v-z = 0;
e, g, i, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1&2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- 2. CMP-SA-PEG, ST3Gal3

```
b, d, f, h, k, m-q, s, u, w, y, z = 0;
a, c, e, g, i, r, t (independently selected)= 0 or 1;
j, l, v, x (independently selected) = 0 or 1;
R = PEG.
```

FIG. 35C

110/498

```
CHO, BHK, 293 cells, Vero expressed EPO a-q, r-u (independently selected) = 0 or 1; v-z = 0.
```

- 1. sialidase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA, ST3Gal3
- 4. CMP-SA-PEG, ST3Gal1

```
a-h, n, q = 1;
i-m, o, r-u (independently selected) = 0 or 1;
v-y = 0; p, z = 0 or 1; R = PEG.
```

FIG. 35D

```
CHO, BHK, 293 cells, Vero expressed EPO a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; v-z = 0
```

1. CMP-SA-PEG, ST3Gal3

```
a-g, n, q = 1; h = 1 to 3;
i, o, p (independently selected) = 0 or 1;
r-u (independently selected) = 0 to 1;
j-m, v-y (independently selected) = 0 or 1;
R = PEG; z = 0.
```

FIG. 35E

111/498

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- 1. GNT's 1, 2 & 5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

a-c, e-g, i, r-t, v-x (independently selected) = 0 or 1; d, h, j-q, u, y, z = 0; R = PEG.

FIG. 35F

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- 1. GNT's 1, 2 & 5, UDP-GlcNAc
- 2. Galactosidase (synthetic enzyme), PEG-Gal-F.

a-c, e-g, n, q-t, v-x, z (independently selected) = 0 or 1; d, h, j-m, o, p, y, z = 0; R = PEG.

FIG. 35G

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Insect cell, yeast or fungi expressed EPO a-d, f, h, j-m, n-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

1. GNT-1, UDP-GlcNAc-PEG

e, i, r, v (independently selected) = 0 or 1; a-h, j-q, s-u, w-z = 0; R = PEG.

FIG. 35H

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-m, n-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- 1. GNT-1, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

a, e, i, r, v (independently selected) = 0 or 1; b-d, f-h, j-q, s-u, w-z = 0; R = PEG.

FIG. 351

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Insect cell, yeast or fungi expressed EPO a-d, f, h, j-m, n-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- 1. GNT-1, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- 3. ST3Gal3, CMP-SA-PEG

a, e, i, j, r, v (independently selected) = 0 or 1; b-d, f-h, k-q, s-u, w-z = 0; R = PEG.

FIG. 35J

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-m, n-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- 1. GNT's 1, 2 & 5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- 3. ST3Gal3, CMP-SA-PEG

a-c, e-g, i-l, r-t, v-x (independently selected) = 0 or 1; d, h, m-q, u, y, z = 0; R = PEG.

FIG. 35K

114/498

```
Insect cell, yeast or fungi expressed EPO a-d, f, h, j-m, n-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1, 2 & 5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- ▼ 3. α2,6-sialyltransferase, CMP-SA-PEG

```
a-c, e-g, i-l, r-t, v-x (independently selected)
= 0 or 1;
d, h, m-q, u, y, z = 0; R = PEG.
```

FIG. 35L

```
CHO, BHK, 293 cells, Vero expressed EPO a-q, r-u (independently selected) = 0 or 1; v-z=0.
```

- 1. sialidase
- 2. CMP-SA, ST3Gal3
- 3. CMP-SA-PEG, ST3Gal1

```
a-h, q, i-o, r-u (independently selected)
= 0 or 1;
v-y = 0; p, z = 0 or 1; R = PEG.
```

FIG. 35M

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CHO, BHK, 293 cells, Vero expressed EPO a-q, r-u (independently selected) = 0 or 1; v-z = 0.

1. CMP-SA-PEG, ST3Gal3

a-h, i-o, q-u (independently selected) = 0 or 1; v-y = 0; p, z = 0 or 1; R = PEG.

FIG. 35N

CHO, BHK, 293 cells, Vero expressed EPO a-q, r-u (independently selected) = 0 or 1; v-z = 0.

1. CMP-SA-PEG, α 2,8-sialyltransferase

a-h, i-o, q-u (independently selected) = 0 or 1; v-y=0; p, z=0 or 1; R=SA-PEG.

FIG. 350

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CHO, BHK, 293 cells, Vero expressed EPO a-q, r-u (independently selected) = 0 or 1; v-z = 0.

1. CMP-SA-PEG, α 2,8-sialyltransferase

a-h, i-o, p-u, v-z (independently selected) = 0 or 1; R = SA-PEG.

FIG. 35P

yeast or fungi expressed EPO r, t, u, v, x, y (independently selected) = 0 or 1; a-m, n-q, s, w, z = 0; $R = (Man)_n$ where n = 1-5, linear or branched.

- 1. mannosidases
- 2. GNT-1, UDP-GlcNAc
- 3. galactosyltransferase, UDP-Gal
- 4. ST3Gal3, CMP-SA-PEG

a, e, j, r, v (independently selected) = 0 or 1; b-d, f-i, k-q, s-u, w-z = 0; R = PEG.

FIG. 35Q

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```
yeast or fungi expressed EPO

r, t, u, v, x, y (independently selected) = 0 or 1;

a-m, n-q, s, w, z = 0; R = (Man)<sub>n</sub>

where n = 1-5, linear or branched.
```

- 1. mannosidases
- 2. GNT-1, UDP-GlcNAc-PEG

e, r, v (independently selected) = 0 or 1; a-h, i-q, s-u, w-z = 0; R = PEG.

FIG. 35R

```
yeast or fungi expressed EPO

r, t, u, v, x, y (independently selected) = 0 or 1;

a-m, n-q, s, w, z = 0; R = (Man)_n

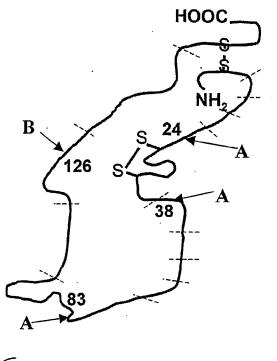
where n = 1-5, linear or branched.
```

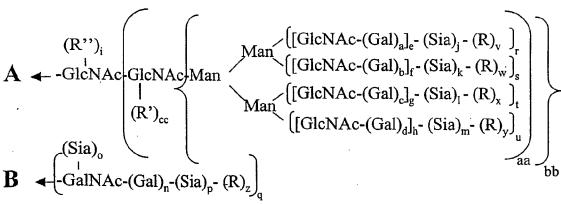
- 1. mannosidase-I
- 2. GNT-1, UDP-GlcNAc
- 3. galactosyltransferase, UDP-Gal
- 4. ST3Gal3, CMP-SA-PEG

```
a, e, j, r, t-u, v, x, y (independently selected)
= 0 or 1;
b-d, f-i, k-q, s, w, z = 0;
(R)<sub>v</sub> = PEG; (R)<sub>x</sub> and (R)<sub>y</sub> = Man.
```

FIG. 35S

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a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. v-z = 0; aa, bb = 1; cc = 0; R = polymer; R" and R' = sugar-polymer or Fuc.

FIG. 35T

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```
yeast or fungi expressed EPO
r, t, u, v, x, y (independently selected) = 0 or 1;
cc, a-m, n-q, s, w, z = 0;
aa, bb = 1;
R = (Man), where n = 1-100, linear or branched.
```

- 1. endo-H
- 2. galactosyltransferase, UDP-Gal-PEG

```
i (independently selected) = 0 or 1;
aa, bb, cc, a-h, j-z = 0; R" = Gal-PEG.
```

FIG. 35U

```
yeast or fungi expressed EPO

r, t, u, v, x, y (independently selected) = 0 or 1;

cc, a-m, n-q, s, w, z = 0; aa, bb = 1;

R = (Man)_n where n = 1-100, linear or branched.
```

- 1. endo-H
- 2. galactosyltransferase, UDP-Gal
- · ▼ 3. ST3Gal3, CMP-SA-PEG

i (independently selected) = 0 or 1; aa, bb, cc, a-h, j-z = 0; R" = Gal-SA-PEG.

FIG. 35V

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```
Insect cell expressed EPO

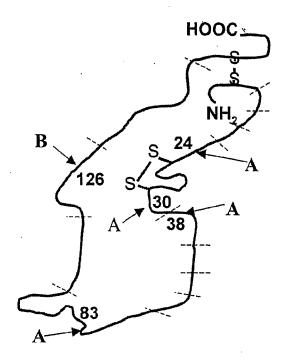
a-d, f, h, j-m, n-q, s, u, v-z = 0;
e, g, i, r, t (independently selected) = 0 or 1;
aa = 1; R" = Fuc.
```

- 1. mannosidases
- 2. galactosyltransferase, UDP-Gal-PEG

```
cc, e, i, r, v (independently selected) = 0 or 1;
bb, a-h, j-q, s-u, w-z = 0; aa = 1; R' = Gal-PEG.
```

FIG. 35W

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$$(Fuc)_{i}$$

$$A \leftarrow -GlcNAc-GlcNAc-Man$$

$$([GlcNAc-(Gal)_{a}]_{e} - (Sia)_{j} - (R)_{v})_{r}$$

$$[[GlcNAc-(Gal)_{b}]_{f} - (Sia)_{k} - (R)_{w})_{s}$$

$$([GlcNAc-(Gal)_{c}]_{g} - (Sia)_{l} - (R)_{x})_{t}$$

$$([GlcNAc-(Gal)_{d}]_{h} - (Sia)_{m} - (R)_{y})_{u}$$

$$([GlcNAc-(Gal)_{d}]_{h} - (Sia)_{m} - (R)_{y})_{u}$$

$$(Sia)_{o}$$

$$(Sia)_{o}$$

$$(Sia)_{o}$$

$$(Sia)_{o}$$

$$(Sia)_{o}$$

a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. v-z = 0; R = polymer.

FIG. 35X

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```
NSO expressed NESP
q = 1; a-i, n, r-u (independently selected) = 0
or 1; j-m, o, p, v-z = 0

1. CMP-SA-levulinate, ST3Gal3,
buffer, salt
2. H<sub>4</sub>N<sub>2</sub>-PEG

q = 1; a-i, j-n, r-y (independently selected) =
0 or 1;
o, p, z = 0; R = PEG.
```

FIG. 35Y

```
CHO, BHK, 293 cells, Vero expressed NESP a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; v-z = 0
```

1. CMP-SA-PEG, α 2,8-ST

```
a-g, n, q = 1; h = 1 to 3;

i, o, p (independently selected) = 0 or 1;

r-u (independently selected) = 0 to 1;

j-m (independently selected) = 0 to 2;

v-y (independently selected) = 1,

when j-m (independently selected) is 2;

R = PEG; z = 0.
```

FIG. 35Z

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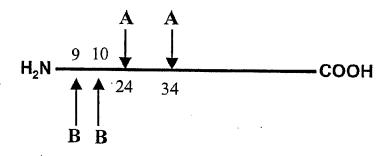
CHO, BHK, 293 cells, Vero expressed NESP a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 to 1; v-z = 0

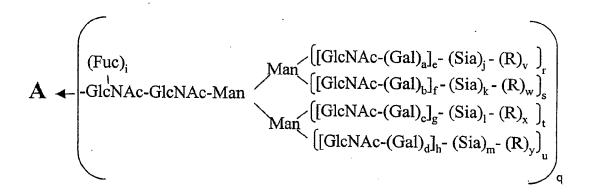
1 CMP-SA, poly- α 2,8-ST

a-g, n, q = 1; h = 1 to 3; i, j-m, o, p, r-u, (independently selected) = 0 or 1; v-z (independently selected) = 0-40; R = Sia.

FIG. 35AA

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$$\mathbf{B} \leftarrow \begin{bmatrix} (\operatorname{Sia})_{o} \\ -\operatorname{GalNAc-(Gal)}_{n} - (\operatorname{Sia})_{p} - (R)_{z} \end{bmatrix}_{aa}$$

a-d, i, n-u, aa (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer, glycoconjugate.

FIG. 36A

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CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h=1; v-z=0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u, aa (independently selected) = 0 or 1;
o, p, z = 0; n, e-h = 1;
v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 36B

```
CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3 & ST3Gal1

a-d, i-m, p-u, aa (independently selected) = 0 or 1;
o, z = 0; n, e-h = 1;
v-y (independently selected) = 0 or 1; R = PEG.

FIG. 36C

126/498

NSO expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0; Sia (independently selected) = Sia or Gal.

- 1. Sialidase and α-galactosidase
- 2. CMP-SA, ST3Gal3
- 2. CMP-SA-PEG, ST3Gal1

a-d, i-m, p-u, z, aa (independently selected) = 0 or 1; n, e-h = 1; o, v-y = 0; z = 1, when p = 1; R = PEG.

FIG. 36D

CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

a-d, i-m, q-y, as (independently selected) = 0 or 1; o, p, z = 0; n, e-h = 1; R = PEG.

FIG. 36E

127/498

CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h=1; v-z=0.

- 1. CMP-SA-levulinate, ST3Gal3, buffer, salt
- 2. H₄N₂-PEG

a-d, i-m, o-y, as (independently selected) = 0 or 1; z = 0; n, e-h = 1; R = PEG.

FIG. 36F

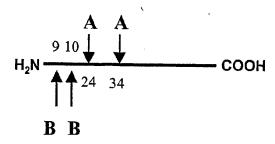
CHO, BHK, 293 cells, Vero expressed GMCSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.

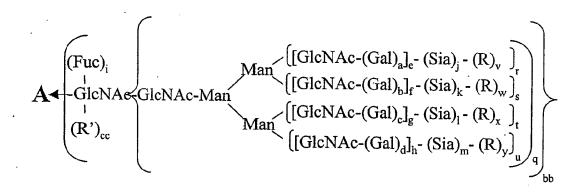
1. CMP-SA, α2,8-ST

a-d, i, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; j-m (independently selected) = 0-20; v-z (independently selected) = 0.

FIG. 36G

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$$\mathbf{B} \leftarrow \begin{pmatrix} (\mathrm{Sia})_{o} \\ -\mathrm{GalNAc-(Gal)}_{n} - (\mathrm{Sia})_{p} - (\mathrm{R})_{z} \end{pmatrix}_{aa}$$

a-d, i, n-u, aa, bb, cc (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose. R'= H, glycosyl residue, modifying group. glycoconjugate.

FIG. 36H

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```
Insect cell expressed GM-CSF.
a-d, f, h, j-m, o, p, s, u, v-z = 0;
e, g, i, n, q, r, t, aa (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, n, q-u (independently selected) = 0 or 1;

j-m = 0; v-y (independently selected) = 1,

when e-h (independently selected) is 1;

R = PEG.
```

FIG. 361

```
Yeast expressed GM-CSF.

a-p, z, cc = 0;

q-y, aa (independently selected) = 0 to 1;

bb = 1; R (branched or linear) = Man, oligomannose;

GalNAc = Man.
```

- 1. Endoglycanase
- 2. mannosidase (if aa = 1).
- 3. Galactosyltransferase, UDP-Gal-PEG

```
a-p, r-z, aa, bb = 0;
q, cc (independently selected) = 0 or 1;
R' = -Gal-PEG.
```

FIG. 36J

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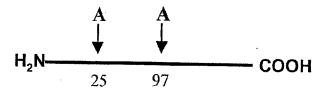
CHO, BHK, 293 cells, Vero expressed GM-CSF. a--m, o-u, aa, bb (independently selected) = 0 or 1; n, v-z, cc = 0.

- 1. sialidase
- 2. CMP-SA, ST3Gal3
- 2. CMP-SA-linker-SA-CMP, ST3Gal1
- 3. ST3Gal3, transferrin

a--m, p-u, z, as (independently selected) = 0 or 1; o, v-y, cc = 0; bb, n = 1; R = transferrin.

FIG. 36K

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$$\mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc\text{-}(Gal)}_a]_e - (\mathrm{Sia})_j - (\mathrm{R})_v \\ [\mathrm{GlcNAc\text{-}(Gal)}_b]_f - (\mathrm{Sia})_k - (\mathrm{R})_w \end{bmatrix}_s \\ [\mathrm{GlcNAc\text{-}(Gal)}_b]_g - (\mathrm{Sia})_l - (\mathrm{R})_x \end{bmatrix}_t \\ [\mathrm{GlcNAc\text{-}(Gal)}_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc\text{-}(Gal)}_b]_f - (\mathrm{Sia})_l - (\mathrm{R})_x \\ [\mathrm{GlcNAc\text{-}(Gal)}_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A}$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 37A

132/498

```
CHO, BHK, 293 cells, Vero expressed IF-gamma. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

1. Sialidase

2. CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 37B

```
CHO, BHK, 293 cells, Vero expressed IF-gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 37C

133/498

```
NSO expressed Interferon gamma.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0;

Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α-galactosidase
- 2. α-Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 37D

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 37E

134/498

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA-levulinate, ST3Gal3,
2. H<sub>4</sub>N<sub>2</sub>-PEG

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 37F

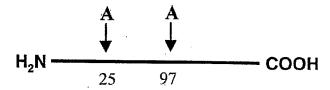
```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1; j-m (independently selected) = 0-20;
v-y (independently selected) = 0.
```

FIG. 37G

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$$\mathbf{A} \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} \\ \operatorname{GlcNAc-Man} \\ (R')_{n} \end{bmatrix} \begin{bmatrix} (\operatorname{GlcNAc-(Gal)}_{a}]_{e} - (\operatorname{Sia})_{j} - (R)_{v} \\ (\operatorname{GlcNAc-(Gal)}_{b}]_{f} - (\operatorname{Sia})_{k} - (R)_{w} \end{bmatrix}_{s} \\ (\operatorname{GlcNAc-(Gal)}_{c}]_{g} - (\operatorname{Sia})_{l} - (R)_{x} \end{bmatrix}_{t} \\ (\operatorname{GlcNAc-(Gal)}_{d}]_{h} - (\operatorname{Sia})_{m} - (R)_{y} \end{bmatrix}_{u} = 0$$

a-d, i, n, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose; R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 37H

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```
Insect or fungi cell expressed IF-gamma.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1;

j-m = 0; v-y (independently selected) = 1,

when e-h (independently selected) is 1;

R = PEG.
```

FIG. 371

```
Yeast expressed IF-gamma.

a-m=0; q-y (independently selected) = 0 to 1; p = 1;

R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

```
a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.
```

FIG. 37J

137/498

CHO, BHK, 293 cells, Vero expressed IF-gamma. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. CMP-SA-linker-Gal-UDP, ST3Gal3
- 2. Galactosyltransferase, transferrin treated with endoglycanase.

```
a-m, q-u (independently selected) = 0 or 1;

p = 1; n = 0;

v-y (independently selected) = 0 or 1;

R = linker-transferrin.
```

FIG. 37K

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h, p = 1; n, v-y = 0.
```

1. CMP-SA-PEG, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h, p = 1;
n, v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 37L

138/498

```
Insect or fungi cell expressed IF-gamma.

a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.

1. GNT's 1 & 2, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, w, y = 0;
e, g, i, r, t, q (independently selected) = 0 or 1;
p = 1; v, x (independently selected) = 1,
when e, g (independently selected) is 1;
R = PEG.
```

FIG. 37M

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

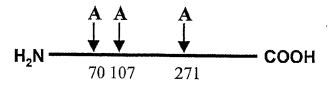
1. CMP-SA-PEG, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;
```

```
a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1; j-m (independently selected) = 0-2;
v-y (independently selected) = 1,
when j-m (independently selected) = 2;
R = PEG.
```

FIG. 37N

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$$\mathbf{A} \leftarrow \begin{bmatrix} \left[\operatorname{GlcNAc-(Gal)}_{a} \right]_{e^{-}} \left(\operatorname{Sia} \right)_{j^{-}} - \left(\operatorname{R} \right)_{v^{-}} \right)_{r} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{b} \right]_{f^{-}} \left(\operatorname{Sia} \right)_{k^{-}} - \left(\operatorname{R} \right)_{w^{-}} \right)_{s} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{b} \right]_{g^{-}} \left(\operatorname{Sia} \right)_{l^{-}} - \left(\operatorname{R} \right)_{w^{-}} \right)_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right)_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right)_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right)_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right)_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right)_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right)_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right)_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{u^{-}} - \left(\operatorname{R} \right)_{u^{-}} \right)_{u^{-}} \\ \left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{u^{-}} - \left(\operatorname{R} \right)_{u^{-}} \\ \left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{u^{-}} - \left(\operatorname{Sia} \right)_{u^{-}} - \left(\operatorname{R} \right)_{u^{-}} \\ \left[\operatorname{GlcNAc-(Gal)}_{d^{-}} \right]_{u^{-}} - \left(\operatorname{Sia} \right)_{u^{-}} - \left(\operatorname{R} \right)_{u^{-}} \\ \left[\operatorname{GlcNAc-(Gal)}_{u^{-}} \right]_{u^{-}} - \left(\operatorname{Sia} \right)_{u^{-}} - \left(\operatorname{R} \right)_{u^{-}} \\ \left[\operatorname{GlcNAc-(Gal)}_{u^{-}} \right]_{u^{-}} - \left(\operatorname{Sia} \right)_{u^{-}} - \left(\operatorname{R} \right)_{u^{-}} \\ \left[\operatorname{GlcNAc-(Gal)}_{u^{-}} \right]_{u^{-}} - \left(\operatorname{Sia} \right)_{u^{-}} - \left(\operatorname{R} \right)_{u^{-}}$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 38A

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```
CHO, BHK, 293 cells, Vero or transgenic animal expressed α<sub>1</sub> antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 38B

```
CHO, BHK, 293 cells, Vero or transgenic animal expressed α<sub>1</sub> antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 38C

141/498

```
CHO, BHK, 293 cells, Vero or transgenic animal expressed alpha-1 antitrypsin.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 38D

```
CHO, BHK, 293 cells, Vero or transgenic animal expressed α<sub>1</sub>-antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

1. CMP-SA-levulinate, ST3Gal3, buffer, salt

 $2. H_4 N_2$ -PEG

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 38E

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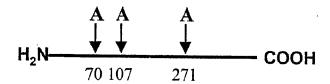
CHO, BHK, 293 cells, Vero expressed α_1 -antitrypsin. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 38F

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$$\mathbf{A} \leftarrow \begin{bmatrix} \left[\operatorname{GlcNAc-(Gal)}_{a} \right]_{e} - \left(\operatorname{Sia} \right)_{j} - \left(\operatorname{R} \right)_{v} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{b} \right]_{f} - \left(\operatorname{Sia} \right)_{k} - \left(\operatorname{R} \right)_{w} \right]_{s} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{b} \right]_{g} - \left(\operatorname{Sia} \right)_{l} - \left(\operatorname{R} \right)_{x} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} \right]_{u} \\ q \\ p \\ \end{bmatrix}$$

a-d, i, n, p-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0;
R = modifying group, mannose, oligo-mannose;
R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 38G

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Insect or fungi cell expressed α_1 -antitrypsin. a-d, f, h, j-m, s, u, v-y = 0; e, g, i, q, r, t (independently selected) = 0 or 1.

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1; j-m = 0; v-y (independently selected) = 1, when e-h (independently selected) is 1; R = PEG.

FIG. 38H

Yeast expressed α_1 -antitrypsin. a-m=0; q-y (independently selected) = 0 to 1; p=1; R (branched or linear) = Man, oligomannose.

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.

FIG. 381

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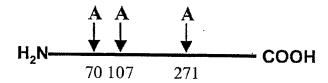
CHO, BHK, 293 cells, Vero expressed α_1 -antitrypsin. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

- 1. CMP-SA-linker-Gal-UDP, ST3Gal3
- 2. Galactosyltransferase, transferrin treated with endoglycanase

```
a-m, q-u (independently selected) = 0 or 1;
p = 1; n = 0;
v-y (independently selected) = 0 or 1;
R = linker-transferrin.
```

FIG. 38J

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$$(Fuc)_{i}$$

$$A \leftarrow GlcNAc - GlcNAc - Man$$

$$(R')_{p}$$

$$(Fuc)_{i}$$

$$(GlcNAc - (Gal)_{a}]_{e} - (Sia)_{j} - (R)_{v}$$

$$[GlcNAc - (Gal)_{b}]_{f} - (Sia)_{k} - (R)_{w}$$

$$[GlcNAc - (Gal)_{c}]_{g} - (Sia)_{l} - (R)_{x}$$

$$[GlcNAc - (Gal)_{c}]_{g} - (Sia)_{l} - (R)_{x}$$

$$[GlcNAc - (Gal)_{d}]_{h} - (Sia)_{m} - (R)_{y}$$

a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. R = polymer; R', R" (independently selected) = sugar, glycoconjugate.

FIG. 38K

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Yeast expressed alpha-1 antitrypsin.
a-h, i-m, p, q = 0;
R (independently selected) = mannose, oligomannose, polymannose;
r-u, v-y (independently selected) = 0 or 1; n, o = 1.

- 1. endoglycanase
- ↓ 2. Galactosyltransferase, UDP-Gal-PEG

```
a-h, i-o, q, r-u, v-y = 0; p = 1.
R" = Gal-PEG.
```

FIG. 38L

```
Plant expressed alpha-1 antitrypsin.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1;
n=1; R' = xylose
```

- 1. hexosaminidase,
- 2. alpha mannosidase and xylosidase
- 3. GlcNAc transferase, UDP-GlcNAc-PEG

```
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, r, t (independently selected) = 0;
q = 1; R' = GlcNAc-PEG.
```

FIG. 38M

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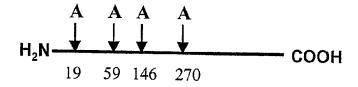
CHO, BHK, 293 cells, Vero, transgenic animal expressed α_1 antitrypsin. a-h, i-o, r-u (independently selected) = 0 or 1; p, q, v-y = 0.

1. CMP-SA-PEG, ST3Gal3

a-h, i-o, r-u (independently selected) = 0 or 1; p, q = 0; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 38N

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$$\mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{a}]_{e}^{-} & (\mathrm{Sia})_{j}^{-} & (\mathrm{R})_{v} \end{bmatrix}_{r}^{r} \\ [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{b}]_{f}^{-} & (\mathrm{Sia})_{k}^{-} & (\mathrm{R})_{w} \end{bmatrix}_{s}^{r} \\ [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{c}]_{g}^{-} & (\mathrm{Sia})_{l}^{-} & (\mathrm{R})_{x} \end{bmatrix}_{t}^{r} \\ [\mathrm{GlcNAc\text{-}}(\mathrm{Gal})_{d}]_{h}^{-} & (\mathrm{Sia})_{m}^{-} & (\mathrm{R})_{y} \end{bmatrix}_{u}^{q}$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 39A

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CHO, BHK, 293 cells, Vero expressed Cerezyme a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 39B

```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. Sialidase
- 2. CMP-SA-M-6-P (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = mannose-6-phosphate
```

FIG. 39C

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```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = Mannose-6-phosphate
```

FIG. 39D

```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- CMP-SA-levulinate, ST3Gal3, buffer, salt
 H N. spacer M & P. and J. A.
- ↓ 2. H₄N₂-spacer-M-6-P or clustered M-6-P

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = M-6-P or clustered M-6-P
```

FIG. 39E

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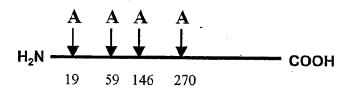
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

1. CMP-SA, α 2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 39F

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$$\mathbf{A} \leftarrow \begin{bmatrix} \left[\operatorname{GlcNAc-(Gal)}_{a} \right]_{e} - \left(\operatorname{Sia} \right)_{j} - \left(\operatorname{R} \right)_{v} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{b} \right]_{f} - \left(\operatorname{Sia} \right)_{k} - \left(\operatorname{R} \right)_{w} \right]_{s} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{b} \right]_{g} - \left(\operatorname{Sia} \right)_{l} - \left(\operatorname{R} \right)_{x} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} \right]_{u} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} \end{bmatrix}_{u} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} \end{bmatrix}_{u} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} \end{bmatrix}_{u} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} \end{bmatrix}_{u} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} \end{bmatrix}_{u} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} \end{bmatrix}_{u} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} \end{bmatrix}_{u} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} \end{bmatrix}_{u} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{Gal}_{d} \right)_{m} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{m} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{m} - \left(\operatorname{Gal}_{d} \right)_{m} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{m} - \left(\operatorname{Gal}_{d} \right)_{m} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right)_{m} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{m} - \left(\operatorname{Gal}_{d} \right)_{m} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{m} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{m} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{m} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} + \left(\operatorname{Gal}_{d} \right)_{m} = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} + \left(\operatorname$$

a-d, i, n, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose; R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG: 39G

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```
Insect cell expressed Cerezyme.

a-d, f, h, j-m, s, u, v-y = 0;

e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1;

j-m = 0;

v-y (independently selected) = 1,

when e-h (independently selected) is 1;

R = PEG.
```

FIG. 39H

```
Yeast expressed Cerezyme.

a-m = 0; q-y (independently selected) = 0 to 1;

p = 1; R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

a-m, p-y=0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.

FIG. 391

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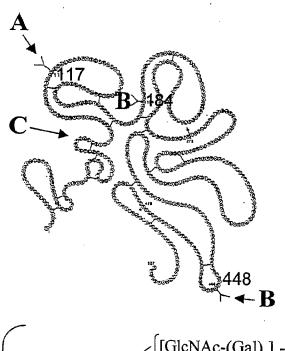
```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

- 1. CMP-SA-linker-SA-CMP, ST3Gal3
- 2. ST3Gal3, desialylated transferrin.
- 3. CMP-SA, ST3Gal3

```
a-m, q-u (independently selected) = 0 or 1;
p = 1; n = 0; v-y (independently selected) = 0 or 1;
R = linker-transferrin.
```

FIG. 39J

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$$(Fuc)_{i}$$

$$\mathbf{B} \leftarrow GlcNAc - GlcNAc - Man$$

$$[[GlcNAc - (Gal)_{a}]_{e} - (Sia)_{j} - (R)_{v}]_{r}$$

$$[[GlcNAc - (Gal)_{b}]_{f} - (Sia)_{k} - (R)_{w}]_{s}$$

$$[[GlcNAc - (Gal)_{c}]_{g} - (Sia)_{l} - (R)_{x}]_{t}$$

$$[[GlcNAc - (Gal)_{d}]_{h} - (Sia)_{m} - (R)_{y}]_{u}$$

$$\mathbf{C} \leftarrow -(\operatorname{Fuc})_{0-1}$$
 $\mathbf{A} \leftarrow -\operatorname{GlcNAc-GlcNAc-Man}$
 $\operatorname{Man} - [\operatorname{Man}]_{0-12}$
 $\operatorname{Man} - [\operatorname{Man}]_{0-6}$
 $\operatorname{Man} - [\operatorname{Man}]_{0-6}$

a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. R = polymer; R' = sugar, glycoconjugate.

FIG. 40A

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```
CHO, BHK, 293 cells, Vero expressed tPA a-g, n = 1; h = 1 to 3; j-m, i, (independently selected) = 0 or 1; r-u (independently selected) = 0 to 1; o, v-y = 0.
```

- 1. Mannosidase(s), sialidase
- 2. GNT1,2 (4 and/or 5) UDP-GlcNAc
- 3. Gal transferase, UDP-Gal
- 4. CMP-SA-PEG, ST3Gal3

```
A = B; a-g, n = 1; h = 1 to 3;
i, r-u (independently selected) = 0 or 1;
o = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG
```

FIG. 40B

```
Insect or fungi cell expressed tPA
A = B; a-d, f, h, j-o, s, u, v-y = 0;
e, g, i, n, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1&2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- **★** 3. CMP-SA-PEG, ST3Gal3

```
A = B; b, d, f, h, k, m, o, s, u, w, y = 0;
a, c, e, g, i, r, t (independently selected) = 0 or 1;
n = 1; j, l, v, x (independently selected) = 0 or 1;
R = PEG.
```

FIG. 40C

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Yeast expressed tPA B = A; i = 0.

- 1. endoglycanase
- 2. Galactosyltransferase, UDP-Gal-PEG

$$A = B$$
; a-n, r-y = 0; $o = 1$; R' = Gal-PEG.

FIG. 40D

Insect or fungi cell expressed tPA A = B; a-d, f, h, j-o, s, u, v-y = 0; e, g, i, n, r, t (independently selected) = 0 or 1.

- 1. alpha and beta mannosidases
- 2. Galactosyltransferase, UDP-Gal-PEG

A = B; a-n, r-y = 0; o = 1; R' = Gal-PEG.

FIG. 40E

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Insect or fungi cell expressed tPA

A = B; a-d, f, h, j-o, s, u, v-y = 0;
e, g, i, n, r, t (independently selected) = 0 or 1.

- 1. GNT's 1&2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

A = B; b, d, f, h, j-o, s, u, w, y = 0; a, c, e, g, i, r, t, v, x (independently selected)= 0 or 1; n = 1; R = PEG.

FIG. 40F

Insect or fungi cell expressed tPA
A = B; a-d, f, h, j-o, s, u, v-y = 0;
e, g, i, n, r, t (independently selected) = 0 or 1.

- 1. GNT's 1 & 2, UDP-GlcNAc
- 2. Galactosidase (synthetic enzyme), PEG-Gal-F.

A = B; b, d, f, h, j-o, s, u, w, y = 0; a, c, e, g, i, r, t, v, x (independently selected)= 0 or 1; n = 1; R = PEG.

FIG. 40G